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Feline Muscular Dystrophy with Dystrophin Deficiency

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This is the first description of a dystrophin-deficient muscular dystrophy in domestic cats. The disorder appears to be of X-linked inheritance because it affected both males of a litter of four kittens. Immunoblotting and immunofluorescent detection of dystrophin showed dystrophin present in control cat muscle but no detectable dystrophin in either affected cat. The feline muscular dystrophy was progressive and histopathologically resembled human Duchenne/Becker muscular dystrophy except for the lack of fat infiltration and the presence of prominent hypertrophy of both muscle fibers and muscles groups in the feline disorder. (Am J Pathol, 1989, 135:909–919)

Dystrophin is a newly defined cytoskeletal protein of muscle fibers that is the normal protein product of the human gene that, when defective, results in X-linked Duchenne/Becker muscular dystrophy (DMD/BMD).[1,2] Consequently, dystrophin deficiency has been shown to be responsible for X-linked muscular dystrophy of mice (MDX)[3,4] and dogs (CXMD).[5–7]

The present report is the first description of a myopathy that also appears to be an X-linked recessive disorder with absence of dystrophin in domestic short hair cats. Included in this report are the cats' conformational and behavioral characteristics, findings pertaining to concentrations of serum muscle enzymes and muscle histochemistry, and results of light and electron microscopy and electromyography. Also described is the study of the dystrophin gene in the affected cats, and biochemical studies of the gene product, dystrophin.

Report of Cases

The subjects, two sibling male cats, one black (cat 1) and one red tabby (cat 2), were born on Nantucket Island, MA in November 1985. Two female siblings were unaffected as was the dam. The presumed sire, a red tabby, was a stray cat that was observed to have a swaying gait that somewhat resembled the abnormal gait of the affected progeny. Although the female siblings did not have signs of myopathy, one had a peculiar, distally located, reducible single-coiled tail, the cause of which has not been investigated.

The dam and each of the progeny were neutered before the diagnosis of myopathy. After diagnosis of progressive myopathy, the cats were donated for further evaluation, euthanasia, and necropsy.

Materials and Methods

At ages 23 or 25 months, the two male sibling domestic short hair cats were given the following examinations: physical, neurologic, ophthalmologic, cardiac (auscultation and EKG, as well as an echogram on cat 2), and electromyography. Laboratory tests performed included feline leukemia ELISA assay; CBC; standard serum chemistry profile analyses; serum creatine kinase (Table 1); T3 and T4 immunoassay; and urinalysis. A coagulation screen was done on cat 1. Cat 2 had two biopsies of the cleidocervicalis muscle at age 14 months.

Cat 1 was necropsied at age 23 months and cat 2 at 25 months. Three young adult castrated male cats serving as normal controls and the affected cats were weighed and measured, and weights of a variety of dissected muscles were compared (Table 2). For the histo-

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Table 1. *Serum Chemistry Results in Two Cats with Dystrophin-Deficient Muscular Dystrophy*

Tests	Cat 1 dates (1987)			Cat 2 dates (1987)			Normal controls
	9/23/87	9/24/87	10/13/87	2/2/87	12/16/87	12/17/87	
Creatine kinase	16,920	NT	32,850	NT	140,800	209,600	<75 U/L
Aspartate transaminase	330	487	558	810	984	1,520	11–39 U/L
Lactic dehydrogenase	367	503	819	1310	NT	NT	63–273 U/L
Alanine transaminase	293	294	221	600	282	295	8–105 U/L

NT, no test.

logic studies of muscles, trapezius and anterior tibialis muscles from affected cats and normal controls were fixed in 10% buffered formalin, embedded in paraffin, and sectioned and stained according to standard techniques with hematoxylin and eosin (H&E) and Masson trichrome stain. Fresh sciatic, ulnar, and peroneal nerves were obtained from cat 2 for teased-nerve preparations and paraffin sectioning for H&E, luxol-fast blue, and Bodian silver staining. Brachial plexus, sciatic nerve, spinal cord, and dorsal and ventral rootlets from cat 1 and 2 were stained with H&E, Bodian, and Masson trichrome. Complete necropsies were done, and sections of all major organs were stained with H&E.

For histochemical studies samples of trapezius and tibialis anterior muscles from the affected cats and normal controls were frozen by submersion in isopentane cooled in liquid nitrogen. Cross sections were cut in a cryostat. Some sections were stained with H & E and modified Gomori trichrome. Other sections were incubated for the histochemical demonstration of the following enzymes activities: succinic dehydrogenase, NADH diaphorase, and adenosine triphosphatase at pH 10.5 and pH 4.6.[8]

For electron microscopy, biopsies of muscle were fixed in glutaraldehyde and embedded in epon.

For dystrophin immunoblot analysis, total muscle proteins were solubilized by placing frozen, pulverized muscle tissue in 20 volumes of sample buffer (10% SDS, 0.1 M Tris, pH 8, 50 mM DTT, and 5 mM EDTA), such that the final protein concentration of the solubilized protein was 50 μg/μl.[2] Two microliter aliquotes (100 μg) were fractionated by electrophoresis on gradient SDS-polyacrylamide gels, transferred to nitrocellulose, and developed for dystrophin.[2] Affinity purified anti-30 kd and anti-60 kd dystrophin antibodies[1] diluted 1:1000 were used. Colorimetric detection of dystrophin was done using alkaline phosphatase-conjugated donkey anti-sheep IgG (Sigma Chemical Co., St. Louis, MO). Monoclonal antibodies against a fast-twitch isoform, the myosin heavy chain,[9] or a fast-twitch isoform of $Ca^{2+}Mg^{2+}$-ATPase[10] were used as controls for muscle protein content of the different immunoblot lanes.

Dystrophin immunofluorescence was done on 4-μm frozen sections[11] with all antibody solutions and washes done with PBS containing 10% horse serum. The primary antibodies were affinity-purified anti-30 kd and anti-60 kd dystrophin antisera,[1] each diluted 1:500. The second antibody was biotinylated anti-sheep IgG (Amersham, Chicago, IL), whereas the third antibody was streptavidin-Texas Red (Bethesda Research Lab, Gaithersburg, MD) both diluted 1:250.

Total genomic DNA was isolated from skeletal muscles of cat 2 and a normal cat. Approximately 5 g of frozen skeletal muscle were homogenized at 4 C in 400 ml of buffer (320 mM sucrose, 10 mM Tris [pH 7.4], 5 mM $MgCl_2$, and 1% Triton X-100) in a Waring blender for 5 minutes at low speed, followed by 2 minutes at high speed. The homogenate was then poured through several layers of cheese cloth, the cloth was rinsed with additional buffer, and the combined solutions were centrifuged in 50 ml tubes for 5 minutes at 3000 RPM (Beckman JA-14 rotor, Palo Alto, CA). After discarding the supernatant, the pellets were resuspended in 5 ml of buffer (24 mM EDTA [pH 8.0] and 75 mM NaCl) and 250 μl of 20% sodium dodecylsulfate (SDS) added with gentle mixing. Next, 1 ml of a 1 mg/ml solution of Proteinase K was added, which was dissolved in the same buffer. The solution was then incubated at 55 C until the solution lost most of its cloudiness (from 1 to 8 hours). After Proteinase K digestion, the DNA was prepared and processed for restriction enzyme digestion and Southern blotting.[12] Probes representative of the entire human DMD cDNA,[13] were used on southern blots containing both PstI and BglI digestions of DNA from a normal control and from cat 2.

Skin tissues obtained from cat 1 at necropsy were cultured and prepared for chromosome analysis according to the air-drying technique of Rothfels and Siminovitch.[14] Metaphase spreads for G-bands were prepared with the trypsin technique of Wang and Federoff.[15] Well-spread metaphase chromosomes were photographed and karyotypes were prepared and analyzed. Karyotypes from ten normal cats served as controls.

Results

Clinical Findings

Cat 1, the black male domestic short hair cat, was considered mildly retarded in that it seldom used the litter box

for either urination or defecation. It was considered to be friendly, affectionate, physically active, and otherwise apparently normal until approximately age 21 months when bilateral symmetrical enlargement of most muscles, except those of the head, was noticed. It slowly acquired cervical rigidity, adduction of the hocks, and, because of muscle stiffness, a falling-down technic when it wanted to laterally recline. It exhibited no problems with eating or drinking, but when under stress its respiratory rate and force were pronounced. Mild anisocoria was seen during the final examination, but the cause was never determined.

Cat 2 was considered abnormal as a young kitten in that it was less active than its three littermates. It had a passive, but friendly and affectionate disposition. At approximately age 6 months, a swaggering gait developed and progressed. Hocks became adducted. The neck enlarged, became rigid, and flexion for eating and grooming became restricted. The circumference of the already large, firm stiff neck increased from 28 to 33 cm between ages 14 and 25 months with no apparent increase in body fat. After 15 months of age, his mouth tended to remain slightly open, probably due to a large tongue that occasionally protruded. Panting episodes were induced by stress; for example, syncope, lasting approximately 30 seconds, and panting occurred when a medicated bath for flea control was given. As was the case with his brother, a falling-down technic was used for lying. Unlike his brother, he always had trouble jumping on chairs and was never able to leap on tables and counters. He lost his ability to curl up when lying and to groom at 8 to 10 months of age because of whole body rigidity. His pupils were reported by the owner to be more frequently dilated than was the case with the other cats, a finding confirmed during examinations, although specific abnormalities could not be found by either ophthalmic examination or later by ocular histopathology. After about 4 months of age, cat 2 would bunny-hop with the rear limbs when running. He often lay on his sternum and ventral abdomen with both rear limbs extended straight posteriorly. Treatment with vitamin E (200 units by mouth daily) and later prednisolone (a total of 175 mg orally on a tapering dosage schedule over 30 days) did not alter the disease course.

No unequivocal neurologic motor or sensory deficit was found on neurologic examination of either cat. Neither cat appeared weak, but both cats tired easily, which may be related to the cause of the easily induced dyspnea. Electrocardiogram findings on cat 1 were normal. An echocardiographic study on cat 2, including both 2-dimensional and M-mode parameters, revealed mild left atrial dilation and mild biventricular dilation, with modest thinning of the left ventricular free wall and septum, and reduced contractility.

Table 2. *Weights of Specific Muscles in Two Cats with Dystrophin-Deficient Muscular Dystrophy and Average Values of Three Control Cats Having Similar Occiput-S3 Measurements*

Variable	Cat 1	Cat 2	Control cats
Biceps brachii	8.5 g	13.2 g	6.7 g
Cranial tibialis	19.0 g	23.5 g	11.0 g
Gastrocnemius	41.0 g	67.0 g	34.6 g
Diaphragm	49.5 g	43.5 g	20.3 g
Body weight	4.55 kg	5.64 kg	3.63 kg
Occiput-S3	42.0 cm	41.0 cm	41.0 cm

Serologic assays for feline leukemia virus antigen by ELISA were negative in both cats. Both cats also had normal CBCs, urinalyses, and chemistry profile values except for enzymes related to striated muscle and liver. Increased enzyme values for aspartate transaminase, lactic dehydrogenase, creatine kinase, and alanine transaminase are listed in Table 1. Coagulation screening tests on cat 1, and thyroxine and triiodothyronine values on both cats were normal.

Electromyography on both cats revealed persistent bizarre high frequency discharges, which waxed and waned after needle insertion into all major muscle groups, accompanied acoustically by traditional "dive-bomber" sounds. There were intermittent spontaneous runs of fibrillation potentials. Nerve isolation and stimulation or blockage procedures were not performed.

Pathologic Findings

At necropsy both cats had pronounced hypertrophy and mild pallor of the skeletal muscles of the tongue, neck, trunk, limbs, and diaphragm. The muscles of the head appeared of normal size. Hypertrophy of muscles was greater in cat 2 (the more severely affected cat). Muscle hypertrophy was most striking in the diaphragm of both cats (Table 2). In cat 2 this caused narrowing of the esophageal lumen at the esophageal hiatus and dilatation (1.4 cm diameter lumen) of the caudal mediastinal segment of the esophagus. The heart of cat 1 weighed 16.5 g and that of cat 2 weighed 18.5 g, with normal weight considered less than 16 g. Both cats had white streaks and patches of up to 0.25 cm in greatest dimension in the left ventricular papillary muscles, left ventricular free wall, and interventricular septum. Cat 2 had a dilated left ventricular lumen. An 0.8 cm, firm yellow lesion was found at the ventral border of one of the anterior lung lobes of cat 2. The same cat had a diffuse red and yellow reticular pattern to the liver.

From both cats muscles that were examined microscopically on H&E stained sections included the tongue,

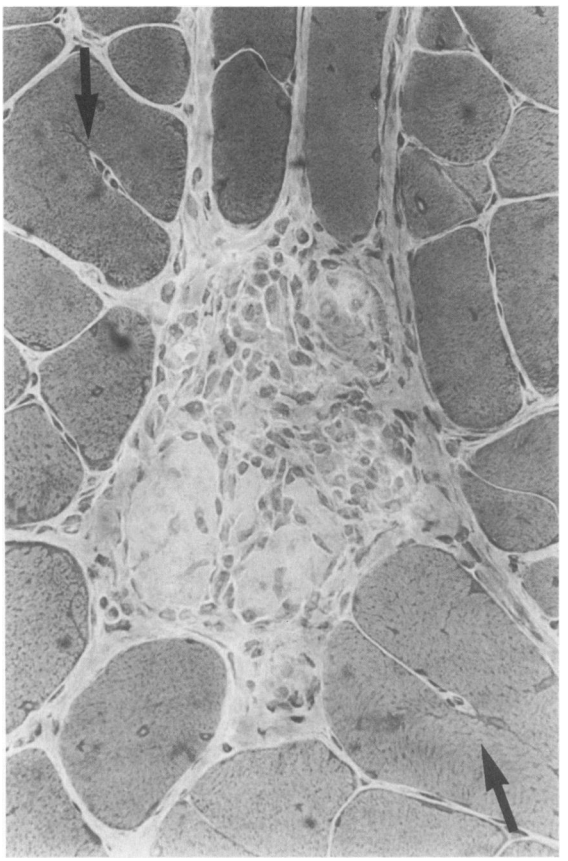

Figure 1. *A group of muscle fibers undergoing necrosis with phagocytosis. Note also the split fibers (arrows) (musculus tibialis anterior, frozen section, modified Gomori trichrome, original magnification ×368).*

temporalis, masseter, diaphragm, intercostal, biceps brachii, cranial tibialis, and gastrocnemius muscles. All skeletal muscles of the 2 cats that were examined showed identical changes. Scattered throughout the muscles were groups of muscle fibers undergoing necrosis and phagocytosis (Figure 1). Other groups of fibers were in various stages of regeneration having basophilic sarcoplasm and large vesicular nuclei with prominent nucleoli (Figure 2). Some other fibers scattered throughout the muscle appeared hypercontracted, being relatively large in diameter, round in contour, and intensely eosinophilic and acid-fuchsinophilic with little morphologic detail visible inside the fiber (Figure 3). A few scattered fibers were mineralized. The remaining muscle fibers varied greatly in diameter, ranging from very small to greatly enlarged. They reached 190 μm in their shorter diameters, whereas the largest muscle fibers in the control cats measured 100 μm. The muscle fibers did not have a rounded or regular polygonal cross section but had a variety of unusual shapes, including ovoid and curved sector. Many groups of fibers formed large ovoid configurations that were absent in the normal control muscles (Figure 4). One of the most striking changes in the muscle was the prominent fiber splitting. There were fibers that had as many as five different splits. In some places ovoid fiber groups appeared to have resulted from the splitting of fibers within a single basal lamina (Figures 4 and 5). Nuclei were frequently centrally located. There was a very mild increase in the endomysial connective tissue in some parts of the muscles but no infiltration with fat. Inflammatory cells were absent. The muscle fibers did not have sarcoplasmic masses or show the ringbinder change. The intramuscular nerves appeared normal, as did the blood vessels.

The histochemical stains showed some fiber-type grouping (Figure 5). Ovoid groups of muscle fibers, as described above, consisted of one or two muscle fiber types. All histochemical types of muscle fibers were present. No other abnormalities associated with muscle fiber enzyme activities were observed. Both large and small muscle fibers were of either histochemical type.

Hearts from both cats contained left ventricular papillary, free wall, and interventricular foci of mineralization and chronic fibrosis that replaced lost myocytes (Figure

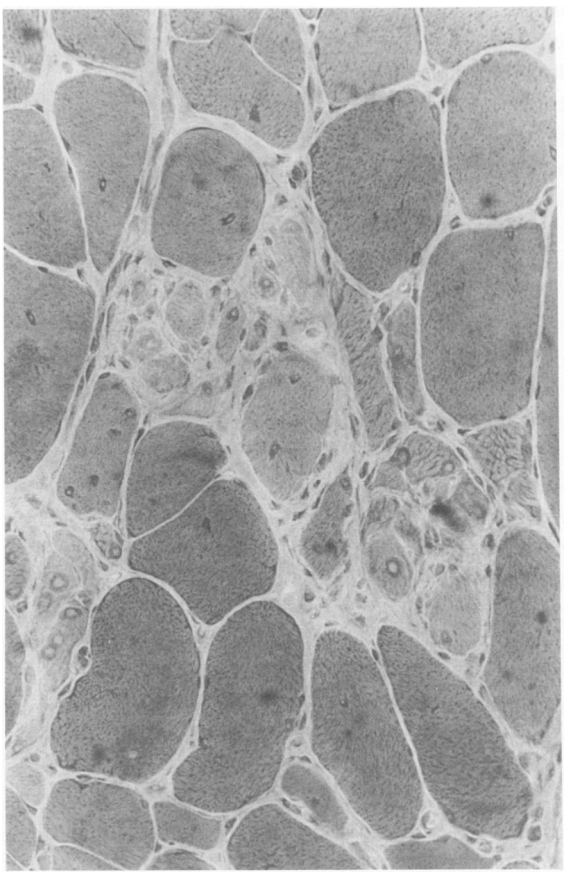

Figure 2. *A group of muscle fibers in the course of regeneration (musculus tibialis anterior, frozen section, modified Gomori trichrome, original magnification ×368).*

6). In addition, there were many scattered brightly eosinophilic myocytes, often with pyknotic nuclei, that were most numerous in cat 2.

Besides the findings in the skeletal muscles and heart, both cats had some microscopic abnormalities in other organs. In cat 1 the liver, although otherwise grossly normal, microscopically showed centrilobular necrosis of individual hepatocytes, which were most frequently adjacent to the central veins. Both kidneys had mild multifocal mineralization in the inner stripe of the outer medulla. In cat 2 there were abnormalities in the liver, adrenal glands, kidneys, and in an anterior lung lobe. The liver had diffuse, severe, centrilobular and mid-zonal hepatocellular swelling and fatty change and mild necrosis of scattered centrilobular hepatocytes. The adrenal glands had bilateral, laminar, and multifocal, swelling, fatty change, necrosis, and mineralization of cortical cells in the zona fasciculata. As was the case with the kidneys in cat 1, there were foci of mineralization in the inner stripe of the outer medullae. In addition, however, there was mild hypoxic nephrosis, with eosinophilic granular casts, that was most numerous in the outer medullae. The gross lung lesion mentioned above was microscopically identified as a chronic granulomatous bronchiolar pneumonia with macrophages and multinucleate foreign body giant cells. No identifiable foreign material was seen, but localized aspiration pneumonia was suspected.

Ultrastructural study of the muscle revealed frequently distended sarcoplasmic reticulum and T system. Mitochondria were often swollen and had irregular shapes and sizes. In some myocytes, they contained calcium granules. The architecture of the sarcomeres was normal in some fibers. In others, there was disruption of the Z-bands and disorganization and disarray of myofilaments to varying degrees. There were a few focal gaps in the plasma membrane, with preservation of the overlying basal lamina. This was seen occasionally in muscle cells that had only minor abnormalities.

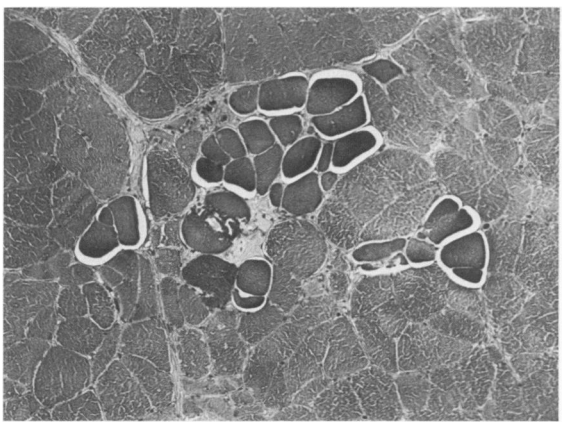

Figure 3. *Musculus trapezius cervicis, transverse section, from cat 2. Dark hyalinized hypercontracted myocytes show grouping and fiber splitting. One fiber (left center) has flocculent necrosis (paraffin section, Masson's trichrome stain, original magnification ×125).*

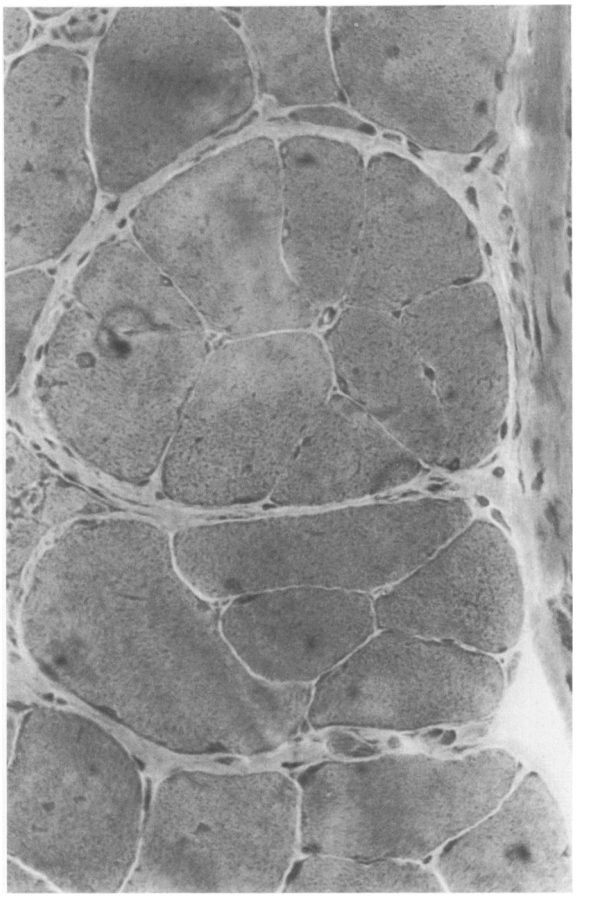

Figure 4. *Groups of muscle fibers forming ovoid configurations delineated by connective tissue (musculus trapezius cervicis, frozen section, modified Gomori trichrome, original magnification ×368).*

Biochemical Analysis of Dystrophin

Dystrophin deficiency was found to be completely specific for Duchenne muscular dystrophy in humans.[13,16] To establish the possible relationship between the muscle disorder in the presently reported cats and human Duchenne muscular dystrophy, autopsied muscle was subjected to both dystrophin immunoblotting and immunofluorescence to determine if the expected quantities of dystrophin were present. Figure 7 shows immunofluorescence analysis of dystrophin in a normal cat (Figure 7A), and one of the affected cats (Figure 7B). Although dystrophin is clearly detected in the normal cat muscle in the expected location (periphery of each muscle fiber), no dystrophin signal was detected in the affected cat.

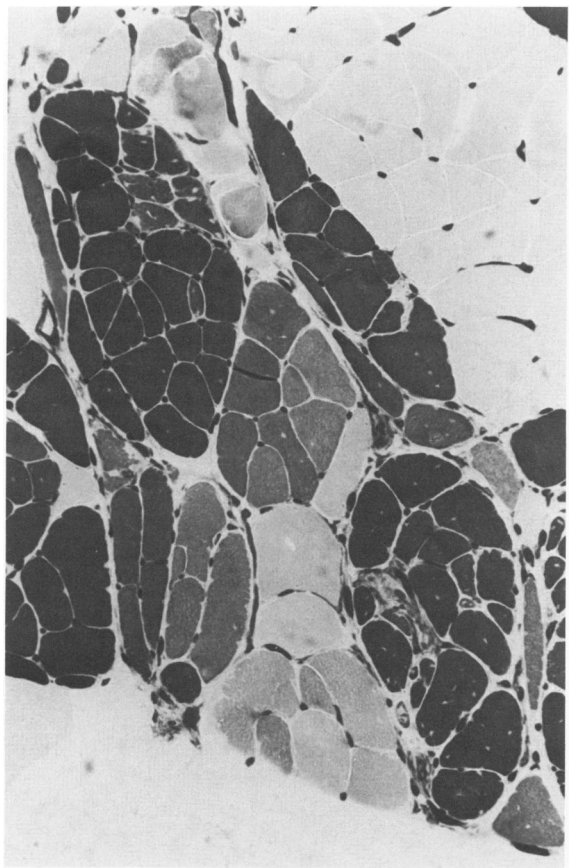

Figure 5. *Grouping of fibers by histochemical type (musculus trapezius cervicis, frozen section, ATPase at pH 10, original magnification ×147).*

Immunoblot analysis of the skeletal muscle of both affected cats gave similar results. Figure 8A shows dystrophin immunoblot analysis of skeletal muscle of one of the affected cats, with normal mouse cardiac muscle and normal dog skeletal muscle in adjacent lanes. Although the normal mouse and dog muscle showed dystrophin at the expected molecular weight (427 kd), no dystrophin was detected in the affected cat. A similar, though less sensitive, immunoblot comparing muscle from both of the affected cats to that from normal cat and mouse muscle is shown in Figure 8B. Again, no dystrophin was detected in either of the two affected cats.

Analysis of Dystrophin Genomic Locus in Normal and Dystrophic Cats

Because the dystrophic cats exhibited a clinical phenotype that was unlike that of severe human Duchenne muscular dystrophy, it was important to ensure that the cats had not sustained a multi-gene mutation (deletion) that was responsible for causing the unusual phenotype. Total genomic DNA was isolated from cat 2 and a normal control cat and subjected to Southern blot analysis. Each of the more than 65 exons of the human Duchenne muscular dystrophy cDNA was used as a probe on a total of six similar Southern blots containing both PstI- and BglI-digested DNA of both cats. As shown in Figure 9, the first (5′ exon of the DMD gene [9C], the middle exons (9B), and last (3′) exons (9A) all appeared to be present in both normal and dystrophic cats. The experiments shown, and others, indicated that all exons of the DMD gene that could be detected in the normal cat were also present in the dystrophic cat. These data indicate that the dystrophic cats did not contain a deletion mutation of the DMD gene.

Discussion

Duchenne muscular dystrophy in humans is considered one of the most common and severe of human genetic

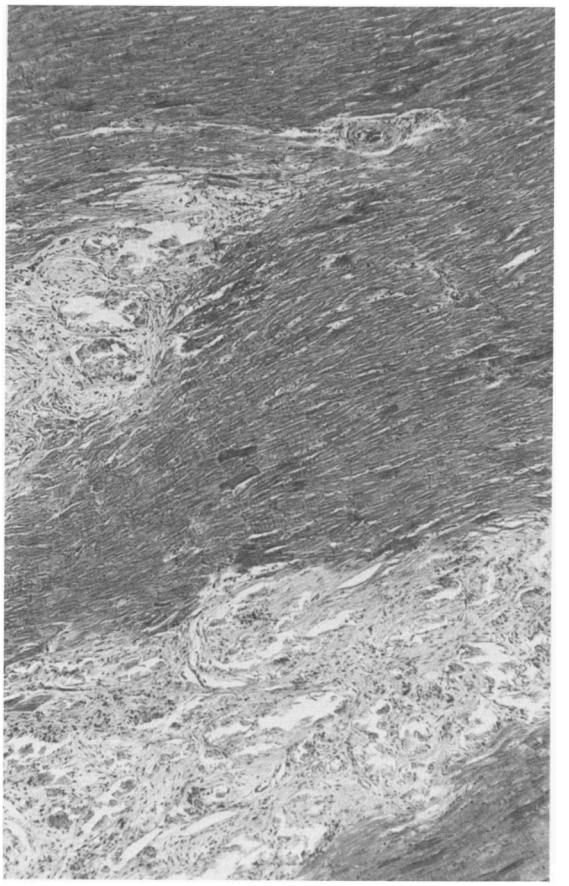

Figure 6. *Left ventricular cardiac muscle from cat 1 showing extensive fibrosis and mineralization. Scattered, short, dark, acid-fuchsinophilic segments of myocytes are degenerating (paraffin section, Masson's trichrome stain, ×50).*

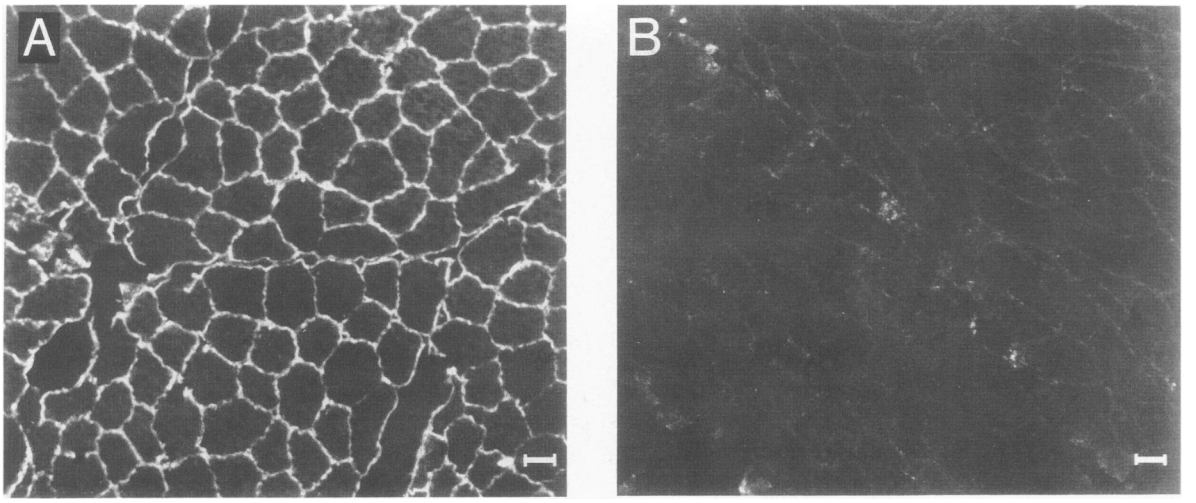

Figure 7. *Dystrophin immunofluorescence in normal and dystrophic cats.* **A:** *Normal cat muscle cross section. Dystrophin immunostaining is seen at the periphery of every muscle fiber.* **B:** *Affected cat muscle cross section. No dystrophin immunostaining signal is evident. All experimental conditions, including photograph exposure time, were identical for A and B. Bar, 50 μm.*

diseases, and has been shown to be the result of dystrophin deficiency.[1,2,16] Dystrophin deficiency was also documented in both mice (mdx)[1] and dogs (CXMD).[7] In this report we describe dystrophin deficiency in domestic cats. The following discussion will both consider the similarities and dissimilarities among each of these four dystrophin-deficient species at the genetic, biochemical, gross pathologic, histopathologic, and clinical levels and examine the pedigree of the cats.

Genetics

The dystrophin gene is on the X-chromosome of most, if not all, placental mammals.[1,17] Indeed, genetic mutations causing dystrophin deficiency have been localized within the dystrophin gene in both DMD-affected humans[13] and mdx mice.[18] Because dystrophin-deficient dogs (CXMD) also inherit the disease as an X-linked trait, the mutation is assumed to be similarly within the X-linked dystrophin gene. Although we describe a very limited pedigree, the inheritance of the dystrophy in the cats in this report also suggest an X-linked recessive inheritance. Given the complete disease specificity of dystrophin deficiency in humans,[2] we concluded that the dystrophin-deficient cats also had a mutation within their X-linked dystrophin gene.

The normal dystrophin gene is composed of over 70 small regions of coding sequence (exons), which together make up the coding sequence for dystrophin. Approximately 65% of dystrophin-deficient human patients (ie, patients with Duchenne muscular dystrophy) show an absence (deletion) of one or more of these exons of the dystrophin gene.[13] In most patients, the deletion of exons results in inactivation of the gene such that the dystrophin protein cannot be produced. In the remaining 35% of human patients, the dystrophin gene appears incapable of producing dystrophin,[2] although the exons appear intact. Thus, it is not known what molecular genetic event causes the inactivation of the dystrophin gene in 35% of human patients. The cats studied in this article, like 35% of human patients, the mdx mouse, and the CXMD dog,[1,7] appeared to have all the exons of the dystrophin gene intact (ie, no deletion of exons was detectable). These data suggest that there was indeed a mutation of the dystrophin gene in the affected cats, although this mutation was not of the deletion type.

Biochemistry

The primary biochemical abnormality the described cats is likely to be dystrophin deficiency for the reasons outlined above. Thus, the cats, DMD-affected humans, CXMD dogs, and mdx mice are all likely to be functionally identical at the biochemical level, ie, all exhibit dystrophin deficiency due to a genetic defect in the X-linked dystrophin gene.

Gross Pathology

The most striking aspect of the cats' gross pathology was the marked hypertrophy of most muscle groups (Tables 2 and 3). Muscle hypertrophy is also evident in the mdx mouse,[19] although it is not as striking as in the described cats. This muscle hypertrophy correlates with the marked muscle fiber hypertrophy in both the cats and mice. Older dystrophin-deficient dogs and humans, however, exhibit

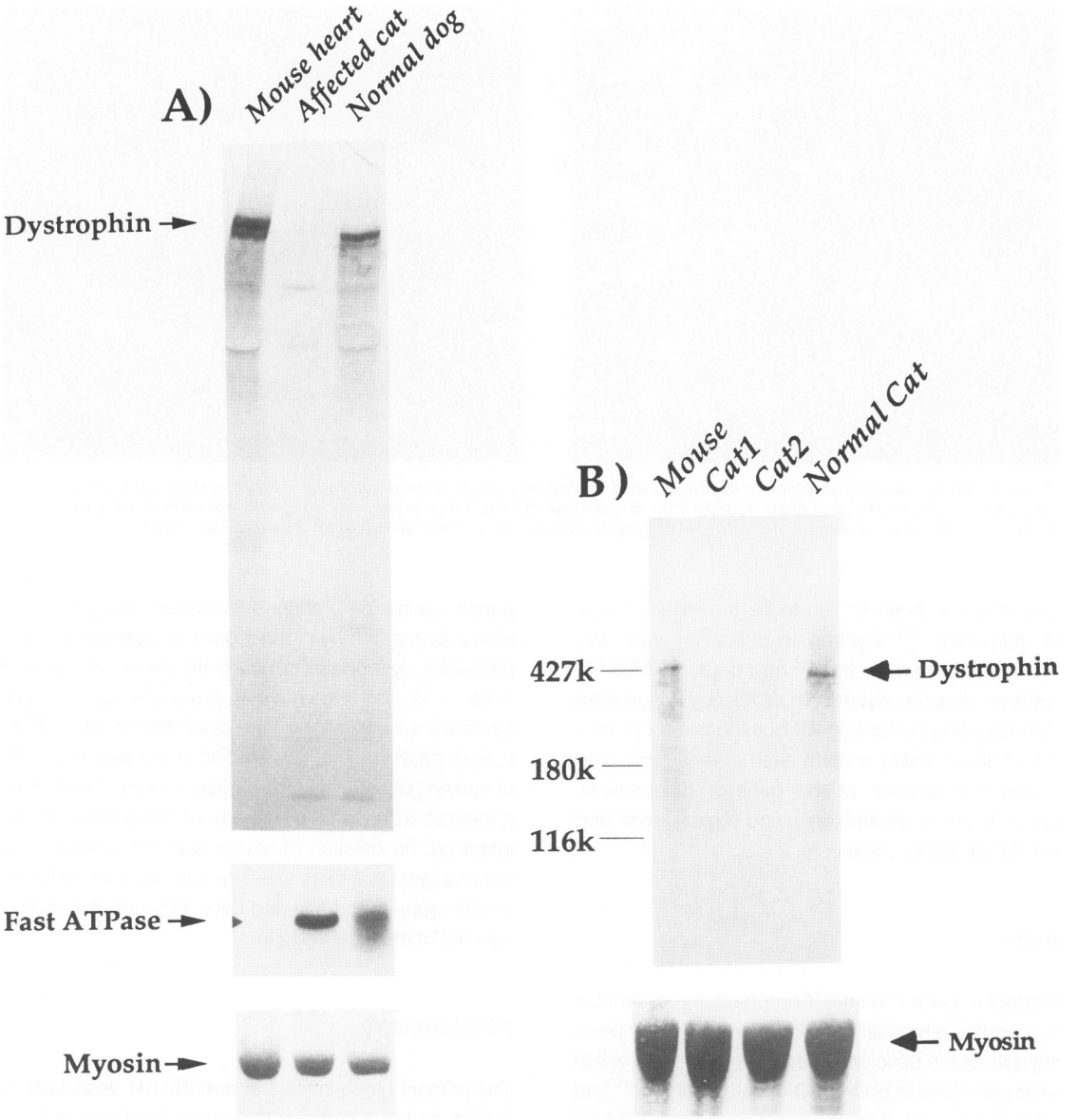

Figure 8. *Dystrophin immunoblot analyses in the myopathic cats.* **A:** *Immunoblot analysis of total muscle protein from normal mouse heart (lane 1), myopathic cat skeletal muscle (lane 2), and normal dog skeletal muscle (lane 3). Dystrophin is detected in the normal mouse and dog muscle, but is undetectable in the affected cat muscle. Parallel blots were incubated with either monoclonal antibody to a fast twitch isoform of $Ca^{2+}Mg^{2+}$-ATPase[10] using monoclonal antibody 'D2'[10], or to a fast twitch isoform of myosin heavy chain.[9] Although the ATPase monoclonal does not detect the ATPase of mouse cardiac muscle (lane 1), it detects similar amounts of the protein in the myopathic cat and normal dog skeletal muscle, verifying the content of muscle protein in the cat lane. The immunoblot shown in this panel has some lanes in common (lanes 1 and 3) with a figure constructed from the same immunoblot in reference 7.* **B:** *Comparison of dystrophin in skeletal muscle of a normal cat, the two affected cats, and in cardiac muscle of a normal mouse. Dystrophin is present in the control muscles (lanes 1 and 4) but is undetected in muscles of both affected cats (lanes 2 and 3). The myosin immunoblot control is shown.*

marked muscle wasting, rather than hypertrophy. Young dystrophin-deficient dogs and humans can exhibit marked hypertrophy early in the disease process.[20,21] Calf muscle hypertrophy is considered a diagnostic hallmark of Duchenne muscular dystrophy. Although calf hypertrophy in DMD has long been considered "pseudohypertrophy," our findings in dystrophin-deficient cats may question whether the hypertrophy in DMD is pseudohypertrophy or true hypertrophy.

Histopathology

Skeletal Muscle

The most obvious manifestation of dystrophin deficiency in all organisms is muscle fiber necrosis (Table 3). Muscle fiber necrosis is evident in neonatal dystrophin-deficient dogs[6] and in humans,[22] but does not begin until approximately 2 to 3 weeks of age in mice.[3,4] Although

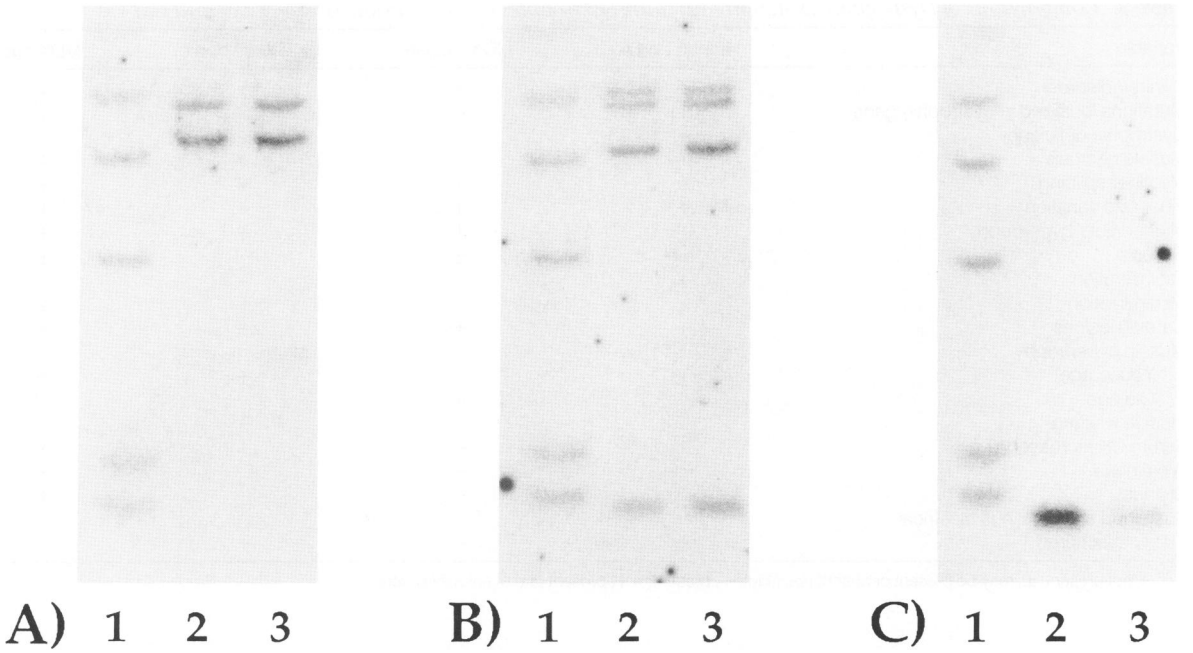

Figure 9. *Dystrophin genomic DNA analysis in normal and dystrophic cats. Shown are examples of the analysis of the dystrophin gene in a normal (lanes 3) and dystrophic (lanes 2) cat. Lane 1 shows molecular weight standards (from bottom: 2.1 kb, 2.3 kb, 4.3 kb, 6.0 kb, and 9.0 kg). Human cDNA probes representing the 5' most exon* **(C)**, *3' most exons* **(A)**, *and central portions* **(B)** *of the DMD gene (lanes 1) were used to detect the corresponding exons in the genomic DNA of the cats. All other exons were also studied in both cats, but are not shown. No abnormality of the dystrophin gene could be detected in the dystrophic cat. This result was consistent with the mouse and dog models of human DMD, which also exhibit no detectable alteration of the dystrophin gene. Approximately 65% of human patients with Duchenne muscular dystrophy exhibit detectable deletions of one or more exons.*

we were unable to study the pathology of the cats at very young ages, we presume that necrosis was probably present from birth or soon thereafter. In all dystrophin-deficient organisms the muscle fiber necrosis continues throughout the life of the organism, accounting for the grossly elevated serum creatine kinase levels (Table 3). An additional histopathologic feature common to all dystrophin-deficient organisms is the large variation in fiber size in all muscles. Much of this variation is likely due to small regenerating fibers. In the cats, however, the large numbers of very hypertrophied fibers were most striking, with individual fiber diameters approaching 200% of normal maximal size. Marked fiber hypertrophy is also seen in dogs, mice, and humans, although it is not as striking as in the described cats. In both dogs and humans, fiber hypertrophy was explained as a compensatory mechanism ("use hypertrophy") due to fiber loss in these organisms. Extensive fiber splitting was also a striking feature of the cats, as it is with all other dystrophin-deficient organisms. The grouping of fiber types, demonstrated by enzyme histochemistry (Figure 5), is believed to be the result of forking of regenerated muscle fibers and has been observed in mdx dystrophy.[3]

Mineralization within the skeletal muscles of the cats was similar to that in CXMD, suggesting that dystrophin is involved with calcium homeostasis. Mineralization was not reported in a 1979 published summary of muscle biopsies of 159 cases of neuromuscular diseases in domestic animals.[23] Mineralization within cardiac muscle of the cats was similar to that in CXMD and DMD.

Myofiber necrosis, fiber splitting, and variation in fiber size appear to be histopathologic features that are common to all dystrophin-deficient animals at all ages, and, as such, could be considered "primary" manifestations of dystrophin deficiency. There are other secondary histopathologic features, namely, fibrosis, fatty infiltration, and muscle wasting, which become prominent with advancing age and in the dystrophin-deficient organisms are pronounced only in humans and dogs (Table 3).

Cardiac Muscle

All dystrophin-deficient organisms appear to manifest cardiac histopathology, although this is minimal and possibly variable in the mdx mouse. The distribution of the cardiac pathology was quite striking in the cat (Figure 6) and was very similar to the very characteristic and unique distribution of the cardiomyopathy in both human DMD[24,25] and CXMD dogs.[20]

Other Organs

The cellular swelling, fatty change, and necrosis in the centrilobular zone of the liver and in the bilateral lamina of the zona fasciculata of both cats were believed to be caused by congestion and hypoxia from decreased car-

Table 3. *Comparisons of Dystrophin-Deficient Muscular Dystrophies of the Human, Mouse, Dog, and Cat*

Variable	Human DMD	MDX mouse	CXMD dog	MDX cat
X-linked disorder	+	+	+	+
Mutations localized in dystrophin gene	+	+	?	?
Dystrophin deficiency	+	+	+	+
Muscle necrosis	+	+	+	+
Myofiber splitting	+	+	+	+
Fiber size variation	+	+	+	+
Marked fiber hypertrophy	+	+	+	+
Fibrosis	++	±	++	±
Fat infiltration	+	−	−	−
Mineralization	+	−	+	+
Cardiac lesions	+	±	+	+
Muscle hypertrophy				
Young age	+	−	+	+
Old age	−	+	−	++
Muscle wasting	+	−	+	−
Serum CK increased	+	+	+	+
Weakness	++	−	++	−
Stiffness	−	−	−	+
Sustained spontaneous electrical discharge	±	−	+	+

−, not present; ±, may be present or absent (variable); +, present; ++, present and eventually severe.

diac function. There was also hypoxic renal tubular necrosis in cat 2 and bilateral tubular necrosis and mineralization in the inner stripe of the outer medulla of both cats. These bilateral and zonal changes also suggest a vascular component in the undetermined pathogenesis of the lesion.

Clinical Manifestations

Despite florid histopathology, neonatal and very young dystrophin-deficient cats, dogs, mice, and humans exhibit no obvious clinical symptoms. The disease is quickly progressive regarding weakness in dogs and humans (Table 3). Specifically, affected humans first show obvious proximal weakness at approximately 5 years of age, and succumb to respiratory or cardiac failure by the mid-twenties. Similarly, dogs show proximal weakness at approximately 3 months of age, and generally succumb to respiratory or cardiac failure by 2 years of age. The cats described in this report did not develop weakness but progressive stiffness, resulting in difficulty in ambulation by 2 years of age; mdx mice develop no obvious weakness or stiffness at any time during their normal life span. The muscle rigidity in the cats was associated with electromyographically evident high frequency activity, often with the dive bomber sounds that are found in myotonic disorders, but that were probably indicative of muscle irritability in these cats. The rigidity of the cats did not worsen after a long rest or exposure to cold and did not disappear with muscle exercise, observations that argue against a true myotonic syndrome.

Mental retardation, seen in 30% of DMD patients, was suggested only by lack of toilet training in cat 1. No explanation was found for the mild anisocoria in cat 1 or the mild bilateral mydriasis in cat 2.

The excessive reaction to stress, such as venipuncture, was manifested in both cats by difficult, rapid, and open-mouth breathing. The unusual stress reaction was most likely related to the large size and increased muscle tone of the muscles of respiration, the large tongue, and poor cardiac function revealed by the cardiac echogram. The histopathologic cardiac and hepatic findings and the high serum alanine transaminase concentration supported the diagnosis of significant heart disease, a condition that would contribute to the pathogenesis of the respiratory signs during stress and to the single episode of syncope.

Pedigree

The detection of muscular dystrophy in the two cats is exciting in that it represents the first documented absence of dystrophin in cats and provides further insight into clinical and pathologic manifestations of dystrophin deficiency. Detection of this rare disorder has also been disappointing in that all members of the family tree, including the mother, maternal grandmother, mother's sisters, and the sisters of the affected brothers were neutered. The father was a stray and was reported to have a somewhat similar gait to that of the affected males. Even if the father was affected by the disorder, he could not through an X-link defect have passed the defect on to his sons. The mother of the affected sons was the likely carrier because

it would be highly unlikely for two unidentical brothers to acquire the same rare genetic mutation. The affected males in this report survived beyond puberty and, if they had not been castrated, might have been physically able to transmit the genetic defect. The surviving members of this family cannot serve as a source for propagation of this disease as an experimental animal model.

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